REMARKS

I. Claim Amendments

By the foregoing amendments to the claims, claims 1 and 14 have been amended to more precisely define the invention. These amendments are supported throughout the application as filed.

The amendments to the claims have been made without prejudice or disclaimer to any subject matter recited or canceled herein. Applicants reserve the right to file one or more continuation and/or divisional applications directed to any canceled subject matter. No new matter has been added, and entry of the foregoing amendments to the claims is respectfully requested.

II. Response to Claim Rejections Under 35 U.S.C. § 103

A. Claims 1-4, 7-9, 12, and 15 have been rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Beresini (1993).

B. Claims 1 and 11 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the combination of Beresini and Zhang (2000).

C. Claims 1, 13, and 14 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the combination of Beresini and Potzsch (1997).

These rejections are respectfully traversed.

As noted in Applicants' prior responses, the present application is directed to methods for determining analyte concentrations in blood plasma when the measurements used to determine the concentration in blood plasma are taken using blood samples rather than blood plasma samples. For this reason, the methods can be used in near-patient settings to determine the concentration of analytes which are, for various reasons, traditionally determined in plasma, but are more conveniently determined in blood at the near patient setting where it may not be possible or convenient to obtain plasma. In other words, the present methods apply to medical laboratory analysis where the desired results are those of plasma analysis, but where the results of blood analysis are either more convenient or the only analysis possible.

Applicants submit that Beresini is not relevant to the subject matter of the present invention. The authors of this reference are interested in the determination of cyclosporine A

(CsA), an analyte that 1) is not, and may never be, an analyte of interest in near patient settings; 2) for determination requires a laborious preanalytical work-up, inconceivable at near patient settings, and; 3) the medical/scientific community is undecided regarding if best to determine in plasma or blood. The study describes a method of determining CsA in anticoagulated blood. However, CsA is not an analyte for which a wealth of clinical data is available for plasma but where the analysis is more conveniently, in near patient settings, performed on blood. Furthermore, the reference does not teach or suggest how to take the results of the CsA determination in blood and from these results determine what the CsA concentration would be in blood plasma. Thus, in contrast to the present invention, Beresini does not address the problem of how to determine analyte concentration in blood plasma from measurements taken using a blood sample.

The Examiner refers in particular to the assay procedure described on page 2236, right column, second full paragraph, of Berensi.

In response, Applicants note the pretreatment of the sample, shown in the 1st full paragraph on the page and column in question:

> **Pretreatment Procedure**
>
> **Calibrators, controls, and samples were dispensed into prelabeled microcentrifuge tubes with a 100-μL positive-displacement pipette. With a 200-μL positive-displacement pipette, methanol was added to each tube to lyse the cells, solubilize the CaA, and precipitate most of the blood proteins. Each tube was closed immediately, and the samples were vortex-mixed for 10 s and allowed to incubate at room temperature for 1 min. The samples were centrifuged in a microcentrifuge (Model 235A; Fisher Scientific, Pittsburgh, PA) at top speed (13 600 × *g*) for 2 min. With a pipetter-diluter (Model 1500, Cavro Scientific Instruments, Sunnyvale, CA; or Modumatic, Tri-Continent Scientific, Grass Valley, CA), a 100-μL aliquot of each supernate was diluted with 200 μL of EMIT Cyclosporine diluent directly into a sample cup. Each cup was immediately capped and the diluted samples were mixed by inversion.**

In the pretreatment, 100 μL of sample (anticoagulated blood) is mixed with 200 μL of methanol to lyse the (blood) cells, solubilize the CsA, and precipitate most of the blood proteins.

The pretreatment results in a relatively clear solution, devoid of most hemoglobin and other components of the blood cells, a solution thus unsuited for any determination which is to correlate with the haematocrit of the original blood sample. Then the assay procedure is described:

Assay Procedure

The assay was performed on the Cobas Mira or Cobas Mira S analyzer (Roche Diagnostic Systems, Montclair, NJ) according to the manufacturer's instructions. The assay is entirely automated and a printout of the results is provided upon completion of each sample. The assay is performed at 37 °C. First, a 75-s incubation of 36 μL of the pretreated sample with 39 μL of water and 155 μL of reagent A allows antibodies to CsA to bind the CsA in the samples. Next, 75 μL of reagent B and 20 μL of water are added. The glucose-6-phosphate dehydrogenase-conjugated CsA competes with the drug in the sample for binding to the antibodies. All components are then allowed to incubate for 175 s. Because the activity of the CsA–enzyme conjugate decreases when bound to antibody, enzyme activity increases with increasing amounts of the drug in the sample. The reaction is monitored spectrophotometrically at 340 nm for 100 s. A calibration curve is generated on the analyzer by a four-parameter, standard nonlinear, curve-fitting program. The curve may be generated at each run or stored from a previous run. The samples are automatically quantified from the existing calibration curve. The calibrators are assayed in duplicate, although a single assay value is sufficient for sample quantification.

In the assay procedure, 39 μL of the pretreated sample is mixed with 250 μL of reagent (155 μL of reagent A, 75 μL of reagent B, and 20 μL of water). The mixture is then incubated, i.e. left as it is, for 175 seconds after which the reaction is monitored spectrophotometrically at 340 nm for 100 seconds. There is only one measurement performed on the mixture and this measurement correlates with the concentration of the CsA. As noted above, it is unlikely that any second measurement that correlates with the haematocrit of the sample is possible as most of the blood proteins, including the hemoglobin and other blood cell components, have been removed in the pretreatment. There is nothing here that leads anyone in the direction of the invention.

The Examiner states that the haematocrit has been measured since haematocrit values of samples are given on page 2238, right column, first full paragraph, lines 1-2. The paragraph in question starts on the left column and continues as stated:

We investigated the effect of hematocrit on CsA recovery. Cells and plasma from normal donors were mixed in selected proportions to generate samples with the following hematocrits: 15%, 26%, 37%, 46%, and 59%. Mean recoveries from these samples ranged from 104% to 115% for 75 μg/L CsA and from 91% to 101% for 425 μg/L CsA (n = 8); this indicated that hematocrit had little effect on CsA quantification. Additionally, choice

The hematocrit values given are those that were derived/generated by mixing blood cells and plasma from normal donors, in the indicated proportions. The hematocrit values appear not to be derived from any measurements made on mixtures of blood and reagent as is recited in the present claims (most likely no results of any measurements correlating to hematocrit have been performed in the study of Beresini, and if so, the measurements have most likely on the blood sample as such, not on any mixture, and most certainly not on mixtures of pretreated sample and reagent). Furthermore, as pointed out in the Applicants' previous responses, there is no reason, nor need, in the assay described by Beresini, for information on hematocrit since the results of the assay of Beresini is practically insensitive to variations in hematocrit - a favorable characteristic of the assay studied by Beresini.

Beresini do not teach or suggest that cyclosporine analysis of blood can provide data of equal medical value as cyclosporine analysis of plasma. On the contrary, Beresini teach/suggest that cyclosporine analysis of blood gives the best clinical data. In Beresini, the problem addressed in the present application, i.e. the problem of how to obtain the level of an analyte in plasma by performing analysis of blood, is not an issue. There is nothing in the report of Beresini that indicates, suggests or implies that two measurements have been performed on a mixture of blood and reagent, one measurement that correlates with the analyte concentration (here CsA) and the other with the hematocrit of the blood.

With regard to Zhang, this reference does not remedy the total deficiency of Beresini to predict, foretell, or make obvious, the invention. Zhang teaches that a separate determination of hematocrit can be performed in a noninvasive way. It may be the case that by practicing the method described by Zang the hematocrit of the blood can become known. However, Zang does not teach or suggest the subject matter of the present claims.

Pötzsch has also been discussed in Applicants' prior responses. This reference cannot be combined with either or both of Beresini and Zhang to aid in reaching the present invention, because Pötzsch only assays plasma.

The Examiner disagrees and states that Pötzsch el at indeed measure hirudin in blood and points at the Material and Methods section, page 375, line 3, where the Examiner finds "blood samples", and on line 5 "citrate". This, however, does not mean that "citrated blood sample" has been subjected to analysis. Pötzsch merely state how "blood samples" were taken, and further describe how the "blood samples" were anticoagulated with "citrate", centrifuged to obtain plasma, and stored in aliquots at -80°C until use. The plasma samples are subsequently thawed and subjected to analysis, as is detailed in the same Materials and Methods section. Pötzsche performed activated partial thromboplastin time (APTT) and ecarin clotting time (ECT) on citrated plasma, not on blood. The subscript to Table 1, page 376, specifies that "pooled normal plasma" has been spiked with hirudin and subjected to ECT assay. No blood is analyzed in the study of Pötzsch. No blood and reagent are ever mixed. No measurements are made on mixtures of blood and reagent.

Applicants also disagree with the Examiner's opinion that Figure 4, page 380, of Pötzsch shows a re-expression of ECT, in seconds, into INR. Instead, Figure 4 shows the plasma ECT of several patients, one ECT at each of two time points for each patient. One time point is before and one is after the start of treatment with phenprocoumon (a derivative of coumarin/warfarin also known under the brand name Marcomar). The assays results of each patient are connected by a line. The treatment with phenprocoumon increases the plasma INR (an expression of the results of a prothrombin time assay). The data in panel A of Figure 4 shows that the plasma ECT of a given person is only marginally affected by considerable upward shift in the plasma INR. ECT appears virtually independent of INR. This is also the conclusion of the data shown in panel B. In panel B, plasmas with various INR are spiked with increasing amounts of hirudin. Plasmas

with various INR are obtained by mixing normal plasma (INR about 1) with a patient plasma with INR 6.0. Figure 4, page 380, panel A and panel B, has nothing to do with converting INR into anything else, it has nothing to do with re-expressing ECT into INR, or *vice versa*. Information given in Figure 4, or elsewhere in the report, does not teach or suggest the possibilities or advantages of converting ECT into INR, or *vice versa* (actually, Pötzsch show that ECT and INR are impossible interconvert because there is hardly any correlation between the two). The possibility and advantage of converting INR, or other non-proportional expressions of an analyte concentration, into proportional expressions, such as PT%, to facilitate necessary calculations to determine an analyte concentration in plasma from the results of analysis on blood, as disclosed in the present application and recited in present claim 14, are totally absent in Pötzsch. This is naturally so, as Pötzsch concern themselves with properties and limitations of certain plasma analysis, not with the problem of obtaining a plasma analyte concentration by analyzing blood.

The effects of variations in sample fibrinogen on ECT determinations is investigated by Pötzsch, results shown in Figure 1, page 377. Effects were found, there is a problem, but Pötzsch do not attempt or suggest any remedy or solution. Nothing is mentioned related to the possibility of including fibrinogen in the ecarin containing reagent, as would have been in line with the present application and claim 13. In addition, Pötzsch point at another potential problem with the ECT assay, one that is caused by variations in sample prothrombin. Again, no solution is suggested. Not even in the discussion section of the report is anything provided, even though the seriousness of the prothombin dependency problem is emphasized.

In summary, the references cited by the Examiner, taken alone or in any combination, do not teach or suggest the subject matter of the present claims. Accordingly, Applicants respectfully request reconsideration and withdrawal of these rejections.

II. Response to Claim Rejections Under 35 U.S.C. § 112, Second Paragraph

At page 4 of the Office Action, claims 1-3 and 14 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for the following reasons:

A. The Examiner has stated that claim 14 is indefinite because the term "INR" is not defined.

In response, claim 14 has been amended by defining the term "INR" as "International Normalized Ratio" (see page 6, lines 1-2, of the present specification).

B. The Examiner has stated that the phrase "the test protocol" lacks antecedent basis.

Claim 1 as amended herein no longer includes the phrase "the test protocol."

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions related to this response, or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney at the below-listed telephone number concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

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